Standard Operating Procedure for Regional Varietal Trial

Authors & Contributors:

Amele Asrat A.Amele@cgiar.org

Introduction

The yam breeding program executes regional varietal trials (RVTs) in collaboration with its national partners in different countries. The objective of the trial is to effectively assess the performances of elite clones from advanced testing pipelines in multiple environments across the participating countries with a view of identifying candidates with superior attributes as new varieties for national release and/or use as parents in crossing program. The RVT often include 14 advanced test clones and 2 check varieties for each species: *Dioscorea rotundata* and *Dioscorea alata*.

Methodology

Site or trial location selection

For each country, trial site selection should be made with high consideration for soil suitability to enhance optimum expression of genotypic potentials genotypes under evaluation. The trial should be conducted at least for two consecutive seasons per site or trial location to assess genotypes' interaction with seasons and locations. Soil and weather data should be collected for each trial site. Other management operations such as weeding, staking, and earthing up should be done at appropriate times.

*Season 1: During the first season, establish RVT trials in locations representative of the targeted production area to assess G x E interaction. However, the number and quality of the seed-yam used might force this first evaluation to be executed a at few experimental stations or testing sites.

*Season 2 onward: Establish RVT trials in more location's representative of the targeted production areas. The RVT trials can be combined with on-station and on-farm experiments, management trials, participatory selection and/or G x E interaction trials.

Test clones and trial establishment

The trials shall be established in a 4x4 simple lattice design in 2 replications across the allocated number of locations. Each plot shall constitute 10 plants in a 1m intra and inter-row spacing and sett weight of 200g shall be utilized. Eight middle plants shall constitute the net plot from which data would be collected. Prepare the full list of clones to be included in the trial along with their pedigree information. The check varieties shall consist of a standard released variety and a local popular cultivar. Each country would use the best local varieties in their respective locations as local checks.

Field Management and Information on Environmental Factors

Field management should follow standard agronomic practices and local procedures to protect the crop from pests and diseases and raise a good yam crop.

The most common agronomic practices to be followed include:

- Depth/height of ridge or mound at least 40cm high
- Planting spacing 1-meter intra row (along ridges/mound) and 1 meter between ridges (inter-row)
- Planting depth of 15 to 20cm deep to prevent setts from exposure sun scotch and rodents
- Weed control: the trial plots should be kept free of weeds throughout the crop cycle to ensure optimum crop growth and performance. Weed completion is a serious problem during the early crop growth (planting to emergence) and this must be controlled with the application of suitable herbicides. Apply a

combination of diuron (a systemic pre-emergent) and glyphosate (a contact) herbicides for effective weed control. Mix diuron and glyphosate at 2.3L and 1.8L, respectively per hectare rate. The application should be done not later than 7 days after planting (DAP) of the yam. Subsequent manual weeding at least twice should be applied to further control weeds in the trial plot.

- Earthing-up or Re-mounding: This activity is required to provide an optimum soil environment for proper development of the roots and tubers. It is normally done during weeding but when there is excessive roots and tubers exposure resulting from heavy rains or rodents it has to be done separately using hoes.
- Staking and Trailing: Yam is a climber and may require proper staking depending on the agroecology for optimum crop growth and performance. A variety trial could be conducted with or without staking depending on the trial objective. If the trial is supposed to be staked, a proper trellising method should be applied to reduce the number of stakes required for the trial area. Staking is normally done about a month after planting at which 90% of the sprouts have emerged in a plot. Regular training of the vines must be carried out at least twice a week for proper twining.
- Harvesting: Yam variety trial could be harvested between 7-9 months after planting depending on the species and maturity duration. In planting operations maximum care and precaution must be given during harvesting to avoid varietal mixture and for proper data collection. Before the harvesting operation, one has to prepare proper labels and arrange other items required for the harvest operation. On harvesting day, the harvestable net plot is carefully marked, and tubers dug out manually. All harvested tubers from the plot are packed on the harvested spot in each plot and the labelled tags for the corresponding plot

assigned for collection of relevant harvest data and proper storage of tubers after data collection.

- Environmental and site descriptors: Record the environmental and site-specific parameters of the location where the trial is being conducted. These include longitude, latitude, altitude, soil, and climate data. Record the climate data (temperature, rainfall, humidity, etc.) for the trial period and also information on soil physical and chemical properties that help to explain spatial patterns among experimental sites in the agro-ecological zones. Data from environmental and site descriptors are important for the interpretation of the results of the trial.

Trial Information Sheet

SITE AND TRIAL INFORMATION			
1. Trial Identification Data:			
Trial code:			
Trial name:			
Year (Day-Month-Year)			
Country:			
Location:			
Agro-ecology:			
Altitude (masl)			
Latitude:			
Longitude:			
Responsible Institution		Responsible person	
Name:		Name:	
Address		Address	
Phone		Phone	

E-mail		E-mail	
2. Trial installation data			
Trial design:			
No. of Entries			
No. of replicates			
No. plant per plot			
No. of rows per plot			
Plot size (m x m)			
Between plants distance (m)			
Between row distance (m)			
Planting density (plants/ha)			
Date of planting			
Date of harvesting			
3. Field data			
Predominant soil texture:			
Organic matter (%):			
Soil pH:			
Total Nitrogen (N):			
Phosphorous [P] (ppm)			
Potassium [K](ppm)			
Field history cropping season t-1			
Field history cropping season t-2			

Field history cropping season t-3			
4. Crop management data			
Fertilizer:			
Name	Date of application	Content	Dose
Weed control/hoeing			
Name of product (mechanical or chemical)	Date of application	Content	Dose
5. Weather data (daily basis)			
Record daily weather data on temperature (max, min, average), rainfall, relative humidity etc using Hobo Remote Monitoring System			

or nearest weather station using a separate data sheet			

Meteorological data and soil analyses are ideally collected to identify spatial patterns among the experimental site's agro-ecological zones. Climatic data may be easily accessible only for on-station trials, whereas availability for other experiments may depend on the proximity of the test site to a meteorological station.

Geographical Information System (GIS) – taking a waypoint

For plant breeders, the strength of spatial data management systems is their capacity to provide information on test locations that can support the analysis of genotype x environment interactions. Ideally, a so-called waypoint is taken with a GIS device to record each trial site's longitude, latitude, and altitude.

Evaluation and data collection

Data should be captured on the following parameters as per the ontology from the Yambase for uniformity:

Number of Tubers Planted [number] (NTP): Count of the tubers planted per plot

Seed Setts (Tubers Planted) Weight [kg](SETW): Record the weight of all seed setts to be planted in a plot in kilogram

Number of Emerged Sprouts/plot [number] (NEP): Count the emerged sprouts (plants) per plot every week starting from the first sprout (plant) on the hill emergence date

Days to First Sprout Emergence [date] (DAYFE): Count the number of days from planting to the first sprout in a plot that emerged

Days to 50% Emergence [date] (DAYSE): Count the number of days from planting to 50% of the sprouts in the plot emerged

Establishment Rate [percentage](STRATE): Record the proportion of established plants per plot from the number of seed setts planted two months after planting. Take count of well-established plants in a plot and calculate the % of plant establishment as the number of established plants in a plot divided by the total number of seed setts planted in a plot multiplied by 100

Date of 50% Flowering [date] (DATF): The date of 50% of the plants in a plot have at least one flower/inflorescence

Flowering Degree/intensity [scale] (FLRI): Scoring when more than 50% of the plants in a plot have flowered as:

Scale	State	Description
0	No bud	No inflorescence: not flowering at all.
1	Aborted bud	Presence of small or rudimentary inflorescences/flowers that can show an abortion or abscission point at the joint of the pedicel.
3	Low	Flowering is scarce with the presence of few flowers (buds, flower buds, flowers, fruits and flower abscissions) per inflorescence and per plant (Less than ten inflorescences per plant)
5	Moderate	Flowering is moderate with some flowers (buds, flower buds, flowers, fruits) per inflorescence and per plant (10-29 inflorescences per plant)
7	Profuse	Profuse flowering with many more flowers (buds, flower buds, flowers, fruits) per inflorescence and per plant (30 to 50 inflorescences per plant).

9	Extremely profuse	Extremely Profuse flowering with abundant flowers (buds, flower buds, flowers, fruits) per inflorescence and per plant (More than 50 inflorescences per plant).

Sex [scale](SEX): Score sex of plant in a plot as 0= Not flowering (Unknown); 1= Female, 2= Male; 3= Female and male (predominantly female); 4= Male and female (predominantly male)

Yam Mosaic Virus (YMV) severity score: This parameter should be evaluated every month from 2 months after planting (MAP) till 6 MAP.

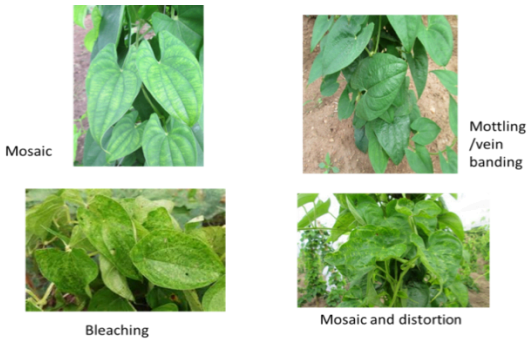

Fig 1. Virus symptom

Virus Severity Score [Scale]: Score virus severity on a 1-5 scale as below

Virus symptoms description on leaf / whole plant	Symptom severity rating scale	Picture
No visible symptoms and virus-negative	1	
Mosaic on most leaves; symptom recovery with time	2	

Mild symptoms on few leaves but no leaf distortion	3	
Severe mosaic on most leaves, leaf distortion	4	
Severe mosaic (bleaching), severe leaf distortion and stunting	5	

Data on severity response should be collected on a plant basis across the net plot. The plant-based data shall be averaged to estimate the area under the disease progress curve (AUDPC) across the six months of evaluation. Estimate mean severity by summing severity scores of >1 in a plot divided by the total number of symptomatic plants.

Yam Anthracnose Disease Severity Score [scale] (YADS): This parameter should be evaluated every month from 2 months after planting (MAP) till 6 MAP. Score anthracnose severity as 1=no visible symptoms of anthracnose disease; 2=few anthracnose spots or symptoms on 1 to ~25% of the plant; 3= anthracnose symptoms covering ~26 to ~50% of the plant; 4=symptom on >51% of the plant; 5= severe necrosis and death of the plant. Estimate mean anthracnose severity by summing severity scores >1 in a plot divided by a total number of symptomatic plants.

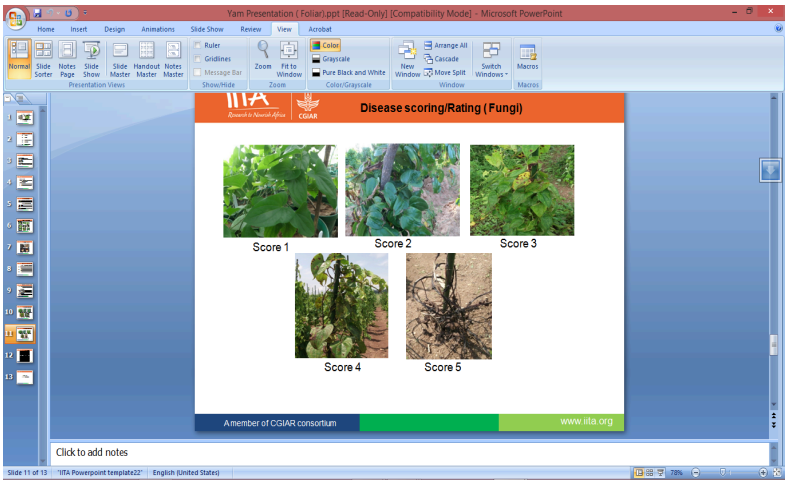

Fig 2. Visual scale for anthracnose scoring

Date of 50% Senescence [date] (DATS): Date when 50% of the plants in a plot are showing signs of foliage senescence

Number of Plants Harvested per Plot [number] (NPH): Count of plants harvested per effective plot

Tuber Yield and yield parameters: These should be collected on a plot basis from the net plot. They include numbers and weights of small (tubers weighing <500 gm each), medium (tubers weighing ≥500 and <1kg) and big tubers (tubers weighing ≥1kg) and rotted tubers. A total number of tubers per plot (count), the total weight of tubers kg per plot (sum of all tuber size categories per plot), average tuber weight kg/tuber (total tuber weight per plot divided by a number of tubers per plot) and fresh tuber yield tons per ha ([total tuber weight per plot (kg) x 10]/plot size (m2) would also be estimated.

Dry Matter and Oxidation: These would be evaluated from samples prepared from plot-based tuber samples. The intensity of Flesh Oxidation after Cutting [scale]: Visual score based on tuber oxidation intensity lasted for the flesh to become or not oxidized as: 1= no oxidation, 3=slightly oxidizing, 5 = highly oxidizing at different time scale: a) at

30 minutes after the cut; b) 180 minutes after the cut. Please refer to the yam trait ontology here; we made slight modifications on the scale. For dry matter determination, please refer to the yam trait ontology.

Boiled and pounded sensory evaluation: These should be evaluated using the participatory varietal selection (PVS) protocol (please see SOP for pounded and boiled sensory evaluation).